Dear Christian,

I called and left a message. Would like to have a telephonic interview and see if the following claims would overcome all the issues of record.

Please give me a call, 202-263-4332.

Thanks much,
Suzannah

Proposed Claims

1. (Withdrawn, Original) An isolated polynucleotide which codes for a polypeptide having the amino acid sequence which is 90 to 100% identical to the amino acid sequences contained in the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10.

2. (Withdrawn, Original) The polynucleotide as claimed in claim 1, selected from the group:

a) polynucleotides comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto,

b) polynucleotides comprising nucleotide sequences which correspond to the sequences from a) within the scope of the degeneracy of the genetic code,

c) polynucleotides comprising nucleotide sequences as in a) which comprise functionally neutral sense mutations,

d) polynucleotides which hybridize with the complementary sequences from a) under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C,

where the polynucleotides code for a cyanide-tolerant nitrile hydratase.

3. (Withdrawn, Original) A polypeptide comprising amino acid sequences which are 90 to 100% identical to the sequences to the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10.

4. (Withdrawn, Original) The polypeptide having the activity of cyanide-tolerant nitrile hydratases as claimed in claim 3, whose remaining activity after conversion of methacrylonitrile in the presence of 20 mM (mM=mmol/l) cyanide ions at 20°C after 30 min is at least 90% of the remaining activity of the same enzyme when it has been categorized for the conversion in the absence of cyanide ions under conditions which are otherwise the same.

5. (Withdrawn, Original) A probe or primer comprising at least 20 consecutive nucleotides from the sequences SEQ ID NO: 1, 4, 6, 9.

6. (Withdrawn, Previously presented) A vector comprising a polynucleotide selected from the group consisting of:

(a) the polynucleotide of claim 1;

(b) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(c) a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(e) a polynucleotide which hybridizes with the complementary sequences of nucleotide sequences SEQ ID NO: 1, 4, 6, or 9 under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C, where the polynucleotides code for a cyanide-tolerant nitrile hydratase.

7. (Withdrawn, Previously presented) A host cell transformed or transfected by the introduction of a polynucleotide selected from the group consisting of:

(a) the polynucleotide of claim 1;

(b) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(c) a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;

(e) a polynucleotide which hybridizes with the complementary sequences of nucleotide sequences SEQ ID NO: 1, 4, 6, or 9 under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C, where the polynucleotides code for a cyanide-tolerant nitrile hydratase
or a vector comprising the polynucleotide.

8. (Withdrawn, Original) A host cell transformed by the introduction of a vector as claimed in claim 6.

9. (Withdrawn, Previously presented) A process for the enzymatic preparation of amides from nitriles, which comprises the following steps:

a) conversion of a compound comprising nitrile groups using a microbial enzyme (polypeptide) which has nitrile hydratase activity and

b) removal of the amide formed,

employing a cyanide-tolerant nitrile hydratase selected from the group consisting of:

(1) the polypeptide of claim 3;

(2) a polypeptide having the activity of cyanide-tolerant nitrile hydratases comprising amino acid sequences which are 90 to 100% identical to the sequences to the sequences SEQ ID NO: 2, 3 and 5 or 7, 8 and 10, whose remaining activity after conversion of methacrylonitrile in the presence of 20 mM (mM=mmol/l) cyanide ions at 20°C after 30 min is at least 90% of the remaining activity of the same enzyme when it has been categorized for the conversion in the absence of cyanide ions under conditions which are otherwise the same,
for the conversion of the nitrile to the amide.

10. (Currently amended) ~~The process as claimed in claim 9, wherein~~ A process for the enzymatic preparation of an amide from a nitrile, which comprises contacting a compound comprising a nitrile group with a polypeptide ~~a host cell transformed or transfected by the introduction of a polynucleotide selected from the group consisting of:~~

(a) ~~a polynucleotide which codes for a polypeptide~~ having ~~the amino acid sequence which is 90 to 100% identical to the amino acid sequences contained in the sequences~~ SEQ ID NO: 2, 3 ~~and~~ or 5 ~~or 7, 8 and 10~~;

(b) encoded by a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, ~~6, 9~~ or nucleotide sequences complementary thereto~~, where the polynucleotides code for a cyanide-tolerant nitrile hydratase~~; or

(c) ~~a polynucleotide comprising nucleotide sequences which correspond to the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, within the scope of the degeneracy of the genetic code, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;~~

~~(d) a polynucleotide comprising the nucleotide sequences SEQ ID NO: 1, 4, 6, 9 or nucleotide sequences complementary thereto, which comprise functionally neutral sense mutations, where the polynucleotides code for a cyanide-tolerant nitrile hydratase;~~

~~(e)~~ encoded by a polynucleotide which hybridizes with the complementary sequences of nucleotide sequences SEQ ID NO: 1, or 4, ~~6, or 9~~ under stringent conditions, where stringent conditions mean washing in 5XSSC at a temperature of from 50 to 65°C~~, where the polynucleotides code for a cyanide-tolerant nitrile hydratase~~

or a ~~vector comprising the polynucleotide, or the~~ microorganism which produces the polypeptide, or a lysate ~~lysates~~ thereof~~, is employed~~.

11. (Currently amended) The process as claimed in claim 10, wherein resting cells of the microorganism are employed.

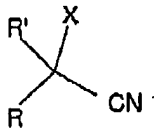
12. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein ~~a purified nitrile hydratase is employed~~ the polypeptide is purified.

13. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein the ~~enzyme~~ polypeptide is derived from microorganisms of the genus Pseudomonas.

14. (Currently amended) The process as claimed in claim 13, wherein the ~~enzyme~~ polypeptide is derived from employed microorganisms of the species Pseudomonas putida or Pseudomonas marginalis.

15. (Previously presented) The process as claimed in claim 14, wherein the employed microorganisms are deposited under the numbers DSM 16275 and DSM 16276.

16. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein ~~compounds of~~ the compound has the general formula (I) or (II)



(I)

$R''-CN$ (II)

where

X is OH, H, alkyl, or NH_2;

R is H, saturated alkyl radical having 1 to 12 C atoms, branched or unbranched, optionally NH_2-substituted, unsaturated alkyl radicals having a double bond and 1 to 12 C atoms, branched or unbranched, cycloalkyl groups having 3 to 6 C atoms, or alkylene radicals substituted by alkylthio groups, where alkyl here corresponds to a C_1 to C_3 radical, and alkylene corresponds to a divalent C_3 to C_8 radical,

R' is H, or an alkyl having 1 to 3 C atoms,

R" is a mono- or binuclear unsatur ated ring having 6 to 12 C atoms, optionally substituted by one or two alkyl groups (C_1 -C_3), Cl, Br, F, or an alkyl nitrile radical having 1 to 6 C atoms,

~~are converted to the corresponding amides.~~

17. (Currently amended) The process as claimed in claim 16, wherein ~~a compound of the general formula (I)~~ the compound is converted in the presence of hydrocyanic acid or a salt of hydrocyanic acid.

18. (Currently amended) The process as claimed in claim 17, wherein the conversion is carried out in the presence of an initial concentration of more than 0.5 mol% cyanide to 3 mol% cyanide, based on the ~~nitrile~~ compound employed.

19. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein 2-amino-4-methylthiobutyronitrile is employed as ~~nitrile~~ the compound.

20. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein 2-hydroxy-4-methylthiobutyronitrile~~, where appropriate present in the reaction mixture from the preparation of this nitrile,~~ is employed as ~~nitrile~~ the compound.

21. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein 2-hydroxy-2-methylpropionitrile is employed as ~~nitrile~~ the compound.

22. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein the amide or the solution comprising the amide is separated from the cells of the biomass, and the amide is hydrolyzed to the corresponding acid.

23. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, wherein the amide or the solution comprising the amide is separated from the cells of the biomass, and the amide is hydrolyzed with alkali metal or alkaline earth metal hydroxides to the salts of the corresponding carboxylic acids.

24. (Currently amended) The process as claimed in claim 23, wherein MHA amide is hydrolyzed with calcium hydroxide, and the calcium salt is obtained.

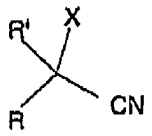
25. (Currently amended) The process as claimed in ~~claim 9~~ claim 10, ~~where~~ wherein

a) ~~microorganisms~~ the microorganism is of the genus Pseudomonas ~~in which isolated polynucleotides which code for polypeptides having the amino acid sequences which are 90 to 100% identical to the amino acid sequences comprised in the sequences with the sequences SEQ ID NO: 2, 3, 5, 7, 8, 10, where the polypeptides have the activity of a cyanide-tolerant nitrile hydratase, enhanced, in particular recombinantly overexpressed, are fermented,~~

~~b) the enzyme produced recombinantly and having nitrile hydratase activity is isolated where appropriate from these microorganisms, or a protein fraction comprising this enzyme is prepared~~ and is fermented to obtain the polypeptide or a protein fraction comprising the polypeptide, and

~~c) the microorganisms according to a) or the enzyme or the fraction comprising the latter according to~~ b) ~~is transferred~~ transferring the polypeptide or the protein fraction into a medium which comprises ~~a compound comprising nitrile groups of the general formulae (I) and (II)~~ the compound.

26. (Currently amended) The process as claimed in ~~claim 10~~ claim 25, wherein ~~compounds of~~ the compound has the general formula (I) or (II)



(I)

$R''-CN$ (II)

where

X is OH, H, alkyl, or NH_2;

R is H, saturated alkyl radical having 1 to 12 C atoms, branched or unbranched, optionally NH_2-substituted, unsaturated alkyl radicals having a double bond and 1 to 12 C atoms, branched or unbranched, cycloalkyl groups having 3 to 6 C atoms, or alkylene radicals substituted by alkylthio groups, where alkyl here corresponds to a C_1 to C_3 radical, and alkylene corresponds to a divalent C_3 to C_8 radical,

R' is H, or an alkyl having 1 to 3 C atoms,

R" is a mono- or binuclear unsaturated ring having 6 to 12 C atoms, optionally substituted by one or two alkyl groups (C_1-C_3), Cl, Br, F, or an alkyl nitrile radical having 1 to 6 C atoms~~,~~

~~are converted to the corresponding amides~~.

27. (Withdrawn, Original) A microorganism of the genus Pseudomonas deposited under the number DSM 16275 or DSM 16276.

28. (Withdrawn, Original) A cyanide-tolerant nitrile hydratase isolated from the strains of the genus Pseudomonas deposited under the numbers DSM 16275 and DSM 16276.